PATHOLOGY

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Fine Needle Aspiration

Pathologic Examination of Tissues

Grossing and processing of image guided biopsies

Number of cassettes

Samples obtained from a single lesion should be placed in a single cassette to allow optimal visualization of histologic relationships. An exception may be the evaluation of specific areas of a lesion (e.g., the margin). It should be understood, however, that evaluation of "margins" by stereotactic differential sampling is an experimental procedure and is currently not considered adequate evaluation of margin status.

Number of levels

Any core biopsy specimen should have at least two levels cut, similar to the practice of cutting prostate biopsies. This still leaves ample tissue for adjuvant testing (e.g. receptor staining) in the vast majority of cases and can significantly add to the diagnostic material.

Documentation of targeting

Documentation of targeting is primarily the responsibility of the physician performing the biopsy. For biopsies performed for microcalcifications the tissue cores should be x-rayed to confirm the presence of calcifications. This information should be made available to the pathologist on every case. The pathology report should mention the presence or absence of calcifications in any case where microcalcifications were the indication for biopsy. For mass lesions the pathologist should recognize if the histologic finding support the mammographic findings. If they don't this should be specifically mentioned in the diagnosis or comment section of the report. An example would be finding normal fattly breast tissue in a case where the target was a well circimscribed solid mass.

Immediate evaluation of adequacy

Imprint preparations for immediate cytologic evaluation may be made to confirm suspicious mass lesions have been sampled. Frozen sections of core biopsies are *strongly* discouraged.

Components of gross description

Necessary items would include type of fixation, confirmation of patient ID and biopsy site. Synoptic or textual formats are acceptable.

Grossing and processing of open biopsies

Orientation

Specimen orientation is an important component of the pathologic examination of breast specimens, particularly therapeutic excisions for cancer (i.e., wide excison, "lumpectomy", segmentectomy, quadrantectomy). In any case where the surgical procedure may be considered therapeutic if in situ or invasive cancer is detected the specimen should be oriented for the dissecting pathologist.
Proper orientation includes enough designations to allow an accurate 3-dimensional determination. This would usually mean at least 2 labels placed by the operating surgeon in additional to the location of the biopsy (i.e., left vs. right). The two labels must not form a single axis. It is recommended that orientation allows the identification of the point nearest the areolar complex to allow sectioning along a "proximal-distal" axis.

Intraoperative Consultation Procedures

1. Specimen x-ray:

Intraoperative specimen x-rays should be performed on all specimens removed for mammographically detected microcalcifications and most specimens removed for mammographic masses. The specimen x-rays should be correlated with preoperative mammographic and needle localization studies to ensure that all abnormal areas have been excised. The procedure may be performed by a radiologist or pathologist.

X-ray of a specimen removed for a mass lesion may be deferred at the discretion of the pathologist, if there is an obvious gross lesion corresponding to the one noted in the preoperative films.

Abnormal microcalcifications must be identified and localized in specimen x-rays and subsequent microscopic sections. An ideal method consists of sectioning the specimen at 2-3 mm intervals, and x-raying and numbering the slices for accurate localization.

2. Inking of specimens:

All intact breast specimens should be inked before incising. The specimen is inked, dipped in Bouin's fixative or other mordant, and blotted before incising. Oriented specimens should be inked in different colors in an attempt to maintain orientation. Margins cannot be adequately assessed on specimens that are already incised or received in fragments.

3. Evaluation of margins:

Intraoperative evaluation of margins remains problematic, and there are no available standard recommendations. Evaluation of specimen X-rays, gross examination, and imprints may be helpful. Frozen section of margins may be utilized in exceptional

circumstances. All methods have significant false negative and false positive rates.

4. Role of frozen section and/or imprints:

Currently, the use of frozen section for the diagnosis of breast lesions is rarely indicated and may compromise the ability to make a reliable final diagnosis on permanent sections. Frozen section for diagnosis should only be performed on masses > 1 cm in size, and only when the result will be used to make an immediate therapeutic decision. Intraoperative cytologic examination (imprint or smear) may be useful to diagnose malignancy, but it may be extremely difficult to distinguish between invasive and in situ carcinoma.

Frozen sections and/or imprints may be useful in evaluating margins as discussed above.

5. Sampling for adjuvant studies:

Fresh tissue may be saved for special studies only for invasive carcinomas > 1 cm. Fresh tissue should not be saved for cases of possible DCIS. Currently, most special studies, including ER and PR determination, can be performed on paraffin-embedded tissue.

Inking of Margins:

Inking of margins should be done on all breast excisions unless received fragmented. Ink should be applied carefully to avoid penetration deep into the specimen. Solutions to aid in "fixing" the ink are encouraged an include vinegar and Bouin's fixative. Multiple colors often aid the microscopist in determining margin status. The overlying principle should be that a pathologist can review the gross description and microscopic slides and be able to distinguish specific margin status.

Sectioning:

Sectioning of breast specimens should always be done in a sequential manner to allow "reconstruction" of the lesion from the microscopic slides. When orientation is given, the axis of sectioning should be documented and, if possible, be along the "areola-distal duct system" axis.

Sampling:

The number of sections submitted varies with the size and character of the specimen and the nature of the underlying neoplastic process. For mammographic abnormalities the entire specimen should be submitted if reasonable. At the minimum, the entire area of mammographic abnormality should be submitted. If high risk or precursor lesions are found (florid hyperplasia, ADH, CIS) the entire specimen should be submitted. For mass lesions, sufficient sections should be submitted to allow accurate determination of tumor type, size, grade, margin status and significant associated features (e.g., lympho-vascular invasion, EIC).

Components and Format of Gross Description:

Either narrative or synoptic formats are acceptable for gross descriptions. Regardless of style, information should include the following:

For specimen-

Fixation type
Tissue(s) included
Intact, fragmented or previously sectioned
Dimensions (including skin dimensions)
Location of biopsy site or tumor
Orientation
Axillary contents (if applicable)
Prosthetic implant (if applicable)
Results of intraoperative consultation

For Tumor-

Size (3 dimensions)
Descriptive features
Correlation with imaging studies
Relation to margins
Other findings (fibrocystic changes, other biopsy sites, etc.)

For Lymph nodes-

Number
Location (if indicated)
Presence of matted nodes

Grossing and processing of mastectomy specimens

Microscopic descriptions

Microscopic descriptions are not required. If, however, this section is omitted it is important that the "Diagnosis" section is well organized and includes all pathologic information that may be necessary for the treating or consulting physician. It is also recommended that a "Comment" section be utilized to document any specific additional aspects of the case.

Diagnosis section

The diagnosis section of the report is critical and it is strongly recommended that it contain all information needed for the treating and consulting physicians. It should be organized in a logical sequence and easy to read and understand. Consistent and appropriate terminology is important and is described below. Information that should be found in the diagnosis include:

Tissue source
Tumor information including:
Histologic type
Histologic grade (see below for grading scheme)
Nuclear grade
Size
Margins
Lymphatic/vascular invasion
Microcalcifications (relation to tumor)
Lymph nodes (# positive/total; size of met; extranodal extension)
Adjuvant studies (receptors, DNA, others)

Standard criteria and terminology

Measurement of Lesion Size

Invasive cancer-

The size of an invasive tumor has prognostic importance and is an important component of staging information. Often a tumor consists of both in situ and invasive areas. When a diagnosis of invasive cancer is made the size given in the diagnosis refers to the *invasive* portion only. The in situ component

is not considered.

For grossly visible tumors, the lesion should be measured in 3 dimensions. The gross measurement should be used in the diagnosis unless microscopic examination shows it to be significantly different (e.g., extension well beyond gross lesion or much of gross lesion is non-invasive disease). For microscopic measurement, the size of the invasive tumor is directly measured. It is important that specimens are sectioned in such a way (i.e., sequentially) that the lesion can be reconstructed, as the most accurate measurement may be found in adding the width of adjacent slices.

In situ cancer-

The measurement of carcinoma in situ is problematic. This is because they only rarely form palpable, visible masses. Several methods for estimating the size (extent) have been suggested, including (1) directly measuring the size of the lesion if it is confined to a single slide; (2) determining the size after submitting the entire specimen for microscopic examination in sequence and in sections of uniform thickness; (3) estimating the percentage of breast tissue involved by DCIS in relation to the total specimen; and (4) reporting the total number of slides examined and the number with DCIS. It is recommended that one of the first two methods described above be used namely,

Directly measuring the size of the lesion if it is confined to a single slide, or
Determining the size after submitting the entire specimen for microscopic examination in sequence and in sections of uniform thickness

Evaluation of Margins

Considerable data indicates that the most significant predictors of local control after breast conservation treatment with lumpectomy and radiation are the status of the surgical margins and the presence or absence of extensive DCIS. The definitions of "positive" and "negative" margins may vary among institutions and studies. The pathology report should clearly state whether tumor is transected at the margin, and if not, how close the lesion is to the nearest margin. The site of involvement should be specified if possible. Blocking of tissue should be directed to evaluate the distance from the edge of the tumor to the margin.

Multifocality

The term "multifocality" has been defined in many ways and often used interchangeably with "multicentricity". Its use should be discouraged and reserved only for those cases of

invasive cancer or DCIS where separate foci are present at least 2 cm apart and it is shown by serial sectioning that there truly are separate foci. Note, several studies using serial subgross sectioning have shown that the vast majority of lesions are continuous and that "multifocality" is usually a product of incomplete sectioning.

Multicentricity

Histologic grading

Every invasive cancer should be graded. Only the invasive component of a tumor should be evaluated when grading. The recommended system to be used is the Elston and Ellis approach to the Scarff Bloom Richardson system, often referred to as the "Modified Bloom Richardson (MBR)" system. This system involves a semi-quantitative assessment of tubule formation, nuclear features, and mitotic index. The criteria are as follows:

Tubule formation-

Definite tubules in at least 75% of tumor area	Score 1
Definite tubules in 10-75% of tumor area	Score 2
Definite tubules in less than 10% of tumor area	Score 3

Tubules must be definite and sharply defined. Spaces from dropout of dead cells are not accepted as tubules.

Nuclear pleomorphism-

Little variation and nuclei appear regular	Score 1
Intermediate of Scores 1 and 3	Score 2
Marked variation with bizarre nuclei	Score 3

This is the least reproducible of the categories. Suggestions to distinguish a nuclear score of 3 include more than 25% of nuclei with size greater than two erythrocytes or coarse clumped chromatin. Nucleoli are seen in both intermediate and high grade nuclei. Multiple nucleoli favors a score of three.

Mitotic rate-

< 10 mitoses per 10 hpf	Score 1
10-20 mitoses per 10 hpf	Score 2
>20 mitoses per 10 hpf	Score 3

Only definite identifiable mitotic figures should be counted. The actual ranges of mitotic figures for each score must be established based on the size of the microscopic field used. Conversion factors are available *(Hum Pathol 1981; 64:1914-1921)*.

Nuclear grading

Some studies show that nuclear grade alone is an important prognostic parameter in invasive cancer. It is also important to determine a nuclear grade for non-invasive lesions because this is an important part of classification. Nuclear grading criteria should be similar to those used as part of the invasive tumor system. An appropriate system is as follows:

Low grade (Grade 1) = Nuclei 1-1.5 rbc in diameter with diffuse chromatin and inapparent nucleoli

Intermediate (Grade 2) = Nuclei 1-2 rbc in diameter with coarse chromatin and infequent nucleoli

High grade (Grade 3) = Nuclei greater than 2.5 rbc in diameter with vesicular chromatin and 1 or more nucleoli

Ductal Carcinoma In Situ (DCIS)

Terminology -

To date there is no wide consensus on the most appropriate classification system for DCIS and this is an area of active research and debate in the literature. A recent Consensus Conference has lead to some recommendations which are considered in the following guidelines. The conclusions of this conference were published simultaneously in ***Cancer* 80,9;1997:1798-1802** and ***The Breast Journal* 8,3;1997:360-364**. This panel stopped short of

recommending a specific classification scheme and rather urged the inclusion of certain pathologic findings in the report. There is, however, much value within a community to use consistent classification scheme to optimize treatment strategies and interpretation of outcome data. For these reasons, the pathology guidelines for this coalition recommends the classification approach expoused by Lagios and others, sometimes referred to as the "Van Nuys classification". This system divides DCIS into 3 categories

High grade (Group 3) - defined by presence of high nuclear grade with or without necrosis

Non-high with necrosis (Group 2) - low or intermediate grade nuclei with comedo-type necrosis.

Non-high grade without necrosis (Group 1) - low or intermediate grade nuclei without necrosis.

The pathologist should understand other classification systems and how they translate into this system. Other correlating terminologies may be mentioned in the report but the above system should be emphasized. If other systems come to the forefront of clinical use then they will be addressed by the pathology group.

The Diagnostic Report -

The above mentioned Concensus Conference recommended that the following tumor characteristics be mentioned in the surgical pathology report of any case of DCIS. Definitions for the features follow:

1. Nuclear grade
2. Necrosis
3. Polarization
4. Architectural pattern(s)

The following tumor information should also be included:

5. Margins
6. Size
7. Microcalcifications
8. Correlation with specimen radiography

Nuclear Grade

Low Grade(NG1). Monotonous in appearance. 1.5-2.0 RBC or duct epithelial cell nuclei diameters, diffuse, fine chromatin, occasional nucleoli and/or mitotic figures. Usually associated with polarization of cells.

Intermediate Grade(NG2). That which is not NG1 or NG3.

High Grade(NG3). Markedly pleomorphic. >2.5 RBC or duct epithelial cell nuclei in diameter. Usually vesicular with irregular chromatin, often prominent nucleoli. May have numerous mitoses.

Necrosis

Definition. Presence of ghost cells and karyorrhectic debris.

Quantification. *Comedonecrosis:* Any central/zonal necrosis within a duct profile, usually in a linear pattern if sectioned longitudinally. *Punctate:* Non-zonal type necrosis.

Cell Polarization

This reflects the radial orientation of the apical portion of tumor cells towards a lumen-like space creating a "rosette" appearance. This appearance is most noticeable in the cribriform pattern but can also be seen in bridges, arcades and micropapillae.

Architectural Pattern

The following patterns are recognized. If multiple patterns are present they should be listed in decreasing order of predominance.

1. Comedo
2. Cribriform
3. Papillary
4. Micropapillary
5. Solid

Note that the term comedo now applies to a pattern of necrosis, not a lesion also characterized by high nuclear grade as it was originally defined. Nuclear grade and architectural pattern are now considered separate reported features.

Extensive intraductal component (EIC)

EIC was originally defined as a tumor where over 25% of the tumor area was DCIS and that DCIS was found beyond the margin of the invasive component. This finding has been associated with higher rates of local recurrence than non-EIC tumors. Recently, some studies have shown that if wide clear margins are obtained, EIC does not effect recurrence rates. Obviously, the concept is that a tumor with EIC has a higher likelihood of residual disease left in the breast following conservative surgery than a non-EIC tumor. When considering a diagnosis of invasive carcinoma with EIC, this concept should be remembered and not take the "25%" figure as an absolute criteria. If a lesion has a *significant* DCIS component *and* DCIS extends beyond the invasive tumor to the extent that it is reasonable to have concern of residual DCIS left in the breast, then a diagnosis of EIC is appropriate. The goal is to provide the treating physicians with information that has importance in constructing a treatment plan.

Atypical Ductal Hyperplasia (ADH) vs. DCIS

The blurred distinction between ADH and DCIS is well documented with significant intra- and interobserver variability. The treatment, however, is radically different. Thus, it is important to make attempts to minimize this variation. This can only be done by adhering to diagnostic criteria as closely as possible. References describing these criteria are available and should be available and understood by any pathologist involved in diagnosing breast lesions. These references include:

Lobular lesions

Adjuvant tests

Hormone receptors

Indications - Estrogen and progesterone receptor testing is indicated in cases of invasive ductal or lobular breast cancer. While of research interest and potential benefit, hormone receptor testing on non-invasive lesion is not indicated on a routine basis.

Methodology - Immunohistochemical methods performed on formalin fixed tissue is the method of choice.

Interpretation - At this point there is no consensus on a preferred method of interpretion of receptor stains. A count of % positive tumor nuclei, a semi-quantitative “scoring” system, or evaluation by image analysis have all shown to correlate with clinical outcome with none being clearly superior. Any of these methods is appropriate. The method used by a lab should be consistent and reproducible, and, if possible, correlated with clinical outcome for validation.

DNA studies

Indications - DNA studies (ploidy, SPF) are indicated in cases of invasive carcinoma. While of research interest and potential benefit, DNA testing on non-invasive lesion is not indicated on a routine basis.

Methodology - Analysis by flow cytometry or image analysis are accepted techniques.

Interpretation -

Substitutions - Some invasive tumors are too small to be analyzed by flow cytometry. In these cases image analysis may be appropriate if available. Another option is immunohistochemical detection of cell proliferation markers such as Ki-67, MIB-1 or PCNA. These markers correlate well with S-phase fraction and mitotic index. Interpretation should be based on established crtieria.

Additional tests

Indications - Many other potential prognostic markers have been evaluated for breast cancer. To date, none have been shown to have significant value and are not indicated outside of a research protocol. A possible exception is Her-2-neu which may have a prognostic role in node positive breast cancer. Methodology and interpretation are not standardized and the use of results should be discussed with the oncologist.

References:

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